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Androgen Receptor Variant–Driven Prostate Cancer II: Advances in Laboratory Investigations

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Abstract

Background: The androgen receptor (AR) is a key prostate cancer drug target. Suppression of AR signaling mediated by the full-length AR (AR-FL) is the therapeutic goal of all existing AR-directed therapies. AR-targeting agents impart therapeutic benefit, but lead to AR aberrations that underlie disease progression and therapeutic resistance. Among the AR aberrations specific to castration-resistant prostate cancer (CRPC), AR variants (AR-Vs) have emerged as important indicators of disease progression and therapeutic resistance.

Methods: We conducted a systemic review of the literature focusing on recent laboratory studies on AR-Vs following our last review article published in 2016. Topics ranged from measurement and detection, molecular origin, regulation, genomic function, and preclinical therapeutic targeting of AR-Vs. We provide expert opinions and perspectives on these topics.

Results: Transcript sequences for 22 AR-Vs have been reported in the literature. Different AR-Vs may arise through different mechanisms, and can be regulated by splicing factors and dictated by genomic rearrangements, but a low-androgen environment is a prerequisite for generation of AR-Vs. The unique transcript structures allowed development of in-situ and in-solution measurement and detection methods, including mRNA and protein detection, in both tissue and blood specimens. AR variant-7 (AR-V7) remains the main measurement target and the most extensively characterized AR-V. Although AR-V7 co-exists with AR-FL, genomic functions mediated by AR-V7 do not require the presence of AR-FL. The distinct cistromes and transcriptional programs directed by AR-V7 and their co-regulators are consistent with genomic features of progressive disease in a low-androgen environment. Preclinical development of AR-V-

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directed agents currently focuses on suppression of mRNA expression and protein degradation as well as targeting of the amino-terminal domain.

Conclusions: Current literature continues to support AR-Vs as biomarkers and therapeutic targets in prostate cancer. Laboratory investigations reveal both challenges and opportunities in targeting AR-Vs to overcome resistance to current AR-directed therapies.

I. Introduction

Prostate cancer is an androgen-dependent disease. Management of patients with advanced prostate cancer often involves androgen-deprivation therapies (ADT) established in 1941 [1]. Under ADT, castrate levels of androgens indicated by circulating testosterone (T) less than 50ng/dL are achieved. Castration-resistant prostate cancer (CRPC) defines disease progression under castrate levels of T. In CRPC, expression level of the androgen receptor (AR) is often elevated, leading to AR activity under reduced androgen levels. In addition, the *AR* gene on the X chromosome may undergo genomic alterations including structural changes and point mutations. These CRPC-specific AR alterations provided a mechanistic explanation for continued dependence of CRPC on AR signaling [2–4]. This important concept in CRPC biology has guided and resulted in successful clinical development of second-generation AR-targeting therapies to treat CRPC, including agents that antagonize AR (enzalutamide, apalutamide, darolutamide) or further suppress extragonadal androgen synthesis (abiraterone, orteronel) [5–17]. The next-generation AR antagonists bind to the AR ligand-binding domain (LBD) with higher affinity than first-generation anti-androgens [6,8], while abiraterone inhibits CYP17A1, a rate-limiting enzyme in the synthesis of adrenal and intra-tumoral androgens [5,7]. Recently, clinical use of these next-generation AR-targeting therapies has been extended to castration-sensitive prostate cancer (CSPC) [9,18,19] and non-metastatic CRPC (nmCRPC) [10–12,20–22].

Androgen receptor variants (AR-Vs) have mRNA sequences that are structurally different from the canonical full-length AR (AR-FL). A total of 22 AR-Vs have been cloned and reported in the literature (Figure 1). The majority of these AR-Vs lack the ligand-binding domain (LBD), the therapeutic target of all existing AR-targeting agents. In preclinical models, some but not all of these AR-Vs mediate constitutively active AR signaling, i.e., their activity is not dependent of the presence of androgens or AR-FL [23]. Among the AR-Vs described to date, AR-V7 remains to be the most extensively evaluated and characterized, and several blood-based tests for AR-V7 have been developed (see companion review). General topics on AR-Vs have been reviewed extensively in the past [23–26]. The intent of the current review is to provide a sequel to a previous review article published in 2016 [24]. Specifically, we will highlight recent preclinical studies covering topics ranging from measurement and detection, molecular origin, regulation, genomic function, and preclinical therapeutic targeting of AR-Vs. We will provide expert opinions and perspectives on these topics. Readers are directed to a companion review focusing on clinical studies related to AR-Vs.

II. Advances in AR-V measurement and detection methods.

Accurate, reliable, and reproducible measurement of AR-Vs is a key requirement for inferring functional and clinical relevance. A variety of detection methods have been developed for the measurement of AR-Vs. These methods differ according to the method of sampling and specific measurement target. Some methods developed for blood-based AR-V7 detection have been analytically validated and implemented for clinical use (see companion review).

Detection by CTC mRNA.

Blood-based detection of AR-V7 in the treatment setting was first reported in 2014 [27]. In this initial report, CTC enrichment was achieved by AdnaTest, followed by RT-PCR-based detection of AR-FL and AR-V7. Following analytical validation, a slightly modified version of the laboratory-developed test was implemented in a CLIA- and CAP-certified laboratory for clinical use [28,29], and another modified version was implemented as a clinical trial test [30]. The AdnaTest employs a simple workflow enabling fast turnaround time at low cost, but with the drawback of requiring sample processing within 24 hours of blood collection. Nevertheless, with careful management this drawback can be overcome. For example, a global biomarker selection trial was conducted by Tokai after implementing three central laboratories in three continents [30]. A recent prospective study further validated the feasibility of conducting the test in the multi-institutional setting involving overnight shipping [13]. While analytical and clinical validity are the two key requirements for clinical implementation, further improvement of the CTC-based test should take into consideration many factors that may also impede or facilitate clinical implementation, including cost and ease of use. Because the number of CTCs detected is always the limiting factor, new technologies to improve efficiency/sensitivity of CTC enrichment may further improve the test. For example, novel microfluidic apparatus or *in vivo* CTC collection methods were designed for CTC isolation and molecular analysis. Using negative depletion microfluidics (CTC-iChip) [31] (NCT01961843) or positive selection microfluidic chip (e.g., IsoFlux) [32], CTC mRNA analysis including analysis of AR-FL/AR-V7 were conducted with digital droplet PCR (ddPCR). An intravascular CTC collection rod with antibody-coated surface (CellCollector, Gilupy, Germany) [33–35], as well as antibody-coated magnetic wire designed for CTC collection directly from blood flow [36], may also help to address the limitation of low CTC numbers. With regard to the limitation posed by low amount of CTC RNA, multiplexing may be the solution. For example, a 27-gene panel (iGene panel) was tested with high-throughput qPCR on Biomark platform (Fluidigm, San Francisco, CA) from CRPC patients receiving docetaxel treatment [37]. These technologies may be used for biomarker development, including the detection of AR-Vs. However, it is challenging to conduct direct comparison of various CTC-based mRNA detection platforms.

Detection in whole blood.

AR-Vs may be detected in whole blood without CTC enrichment. Using blood collection tube containing additives for the purpose of stabilizing intracellular RNA (e.g. PAXgene Blood RNA tube), total RNA from peripheral blood were prepared for AR-V7 detection [38–41]. However, mainly due to RNA contamination from large amount of leukocytes, the

tumor-cell origin of AR-V7 will need to be validated especially when the measured signals are low. In addition, it is important to note that measurement of AR-FL is no longer possible in whole blood samples because non-CTC cells such as regulatory T-cells and macrophages have low-level of AR expression [42,43].

Detection in exosomes.

Exosomal RNA from extracellular vesicles in plasma represents another source for AR-V detection. Using exosomes, AR-V7 was detected by ddPCR using TaqMan probe with high sensitivity (at 2 copies/mL blood) and shown to be a strong predictive marker for enzalutamide/abiraterone resistance in mCRPC patients [44]. In another report, extracellular vehicles (EVs) from urine were used in detecting AR-V7 [45]. Again, studies designed to conduct head-to-head comparisons are important but challenging to implement in the treatment setting.

Tissue Detection by RISH.

PCR-based detection methods described above are in-solution methods that generally do not capture cellular heterogeneity and the morphological context in tumors. In-situ detection methods such as RNA in situ hybridization (RISH) and immunohistochemistry (IHC) (see below) have been developed. The use of RISH for AR-V7 detection was first reported in 2014 [27]. Subsequently, different types of probes, including padlock probes, modified branched DNA probe, or junction-specific probes with enhanced sensitivity and specificity were developed to evaluate AR-FL and AR-Vs in FFPE tissue biopsy and isolated CTCs [34,46–48]. In these studies, higher AR-V7 levels were associated with poorer response to AR-targeting therapies in mCRPC [47,48].

Tissue Detection by IHC.

Recent studies on in-situ detection of AR-V7 protein by IHC focused on newly developed and validated antibodies [48–53]. Using matched CSPC and CRPC tissue samples, nuclear AR-V7 protein detected by a rabbit AR-V7 monoclonal antibody (EPR15656; Abcam, Burlingame, CA) was associated with poor prognosis of CRPC patients after abiraterone or enzalutamide treatment [51]. Following detailed characterization in clinical cohorts [13,49,54], the EPIC CTC AR-V7 IHC test (Epic Sciences, San Diego, CA) initially developed using the same anti-AR-V7 antibody was recently implemented for clinical use (see companion review). In tissue-based studies, the use of the RM7 (RevMab) antibody further established CRPC-specific expression AR-V7 [48,50,55]. Interestingly, AR-V7 protein expression was detected using this well-validated antibody in a subset of cells in small cell prostate carcinoma and some salivary ductal carcinoma specimens from untreated female patients [52,56]. These recent studies on AR-V7 protein expression further supported that AR-V7 expression arises specifically in a low androgen environment.

Tissue Detection by NGS.

AR-Vs can be detected in clinical specimens by RNA-seq [27]. In Antonarakis et al, two autopsy specimens from mCRPC patients with positive CTC AR-V7 underwent RNA-seq analysis. Using number of reads spanning the exon 3/cryptic exon (CE3) and exon 7/exon 8

junctions as surrogate expression values for AR-V7 and AR-FL, respectively, the AR-V7/AR-FL ratios in the two samples were 25.8% (139/539) and 12.1% (151/1248), in line with AR-V7/AR-FL ratios (median 21%, range 1.8% to 208%) estimated from RT-PCR-based CTC AR-V7 test reported in the same study. Using a slightly different method, the median AR-V7/AR-FL ratio from a larger-scale study of CRPC tissues was estimated at ~5% [57]. The lower % reported in tissue-based studies is expected and does not contradict with our initial report in unselected CRPC tissues [58] or the ratios reported in CTC AR-V7 positive cases, because higher ratios reported in the Antonarakis study [27] excluded CTC-AR-V7 negative cases (no ratios can be calculated in AR-V7 negative cases). In addition, RNA-seq in primary hormone naïve prostate cancers also detected AR-V7 at slightly lower AR-V7/AR-FL ratio [59]. This is not unexpected either, as primary PC tissues are known to have low levels of AR-V7 detected by RT-PCR [58]. However, we previously posited [24] that since AR-FL is also overexpressed in CRPC, a ratio of AR-V7/AR-FL at 10%, under the assumption of 10-fold overexpression of AR-FL in CRPC tissues, would bring the absolute level of AR-V7 equivalent to the level of AR-FL detected in primary hormone naïve prostate cancers tissues. This interpretation also explained why AR-V7 protein is not detected in primary tissues [50].

Recent AR-V studies using whole-genome sequencing.

Recent studies have used whole-genome NGS to examine AR for DNA structural alterations and RNA expression. Henzler et. al. [60] evaluated 30 soft tissue metastases from 15 rapid autopsies for AR structural changes and AR-V expression. In this study, diverse AR-genomic structural rearrangements (AR-GSRs) including deletion, duplication, inversion and translocation were observed in 10/30 metastases (6/15 patients). This study discovered many novel AR-Vs driven by AR-GSR. Cloned AR-Vs are depicted in Figure 1. A pilot study by De Laere et. al. [61] applied NGS to CTC and cell-free DNA (cfDNA) from mCRPC patients. In this study, cfDNA was evaluated by low-pass whole-genome sequencing and targeted sequencing of 112 genes including all coding exons and non-repetitive intronic regions of AR, and expression levels of AR-FL and AR variant (AR-FL, AR45, AR-V1, AR-V2, AR-V3, AR-V5, and AR-V7) was interrogated by amplicon sequencing from multiplex junction–specific PCR using cDNA derived from CTCs. In 30 CTC samples from 26 mCRPC patients, 15/26 (57.7%) patients were AR-V-positive with AR-V7 being the most frequently detected variant (12/15 patients), followed by AR-V3 (11/15), AR45 (10/15), AR-V9 (6/15), AR-V1 (5/15), AR-V2 (3/15), and AR-V5 (3/15). In this study, 50% (15/30) of cfDNA samples had an intra-AR structural variation, and 14 of these 15 samples were positive for AR-Vs. Within the small cohort of patients treated with abiraterone or enzalutamide, 15/26 (57.7%) were AR-V-positive, and positive patients were either resistant (13/15) or moderately responsive (2/15) to abiraterone or enzalutamide.

Kallio et. al. also applied targeted AR DNA-seq and RNA-seq to examine AR-Vs and other AR aberrations in CSPC, CRPC, mCRPC, BPH, and noncancerous tissues [62]. AR-V3, AR-V7, and AR-V9 were most frequently detected and co-expressed AR mRNA variants in mCRPC, and these variants were also detected in BPH and hormone-naïve primary tumors with lower abundance and frequency, although corresponding protein expression (in BPH and hormone-naïve samples) was not shown. AR mutations and copy number changes were

only detected in locally recurrent and metastatic CRPC specimens but not in untreated patients. Non-recurring AR-GSRs (i.e., breakpoints are unique to each sample) were also specifically detected in 5/30 mCRPC. In this study, no AR-GSR co-existed with AR missense mutations in mCRPC [62]. In addition, no definitive association was found between these AR-GSRs and AR-Vs, in line with the finding by Henzler et al [60].

Due to limitation of short-read NGS sequencing by Illumina, the contribution of AR GSR to AR-V generation remains to be thoroughly investigated. To date, only one study used a long-read sequencing method (PacBio sequencing platform) to characterize AR variants. In this prospective biomarker study (PROMOTE trial, NCT01953640), Kohli et. al. performed targeted AR RNA-seq, whole transcriptome sequence, and long-read sequencing of AR 3' RACE (rapid amplification of cDNA ends) products in cell lines, patient-derived xenograft, and metastatic CRPC biopsy tissue samples [63]. Expression of AR and AR-Vs was also measured by qRT-PCR in CTC samples. This study found that the 3' UTR of AR-V9 included cryptic exon 3 (CE3) of AR-V7, and high-level of baseline AR-V9 was associated with resistance to abiraterone [63].

III. Molecular origin and regulation of AR-V expression

It is well established that expression of AR-Vs are regulated by androgens [64–69]. Since our last review in 2016 [24], many studies have been published that are relevant to the topic of molecular origin and regulation of AR-V expression. We will summarize the current literature on this topic in three general categories: 1) studies focusing on the role of genomic alterations; 2) studies focusing on the role of RNA splicing; and 3) studies demonstrating the role of other co-factors and signaling pathways on regulating AR-V expression. The findings from studies focusing on splicing factors and related regulators are summarized in Figure 2. Readers are directed to a recent review [70] for further reading on the specific topic of AR-V regulation, the role of genomic structural alterations, and the requirement of low androgens for AR-V7 generation.

Role of genomic alterations.

AR genomic structural rearrangements (GSRs) underlie the generation of some of the AR-Vs. As discussed in the section "Recent AR-V studies using whole-genome sequencing", AR GSRs may also contribute to the generation of AR variants [60–63]. In an interesting model proposed by Henzler *el. al.*, successive structural alterations may occur on the same AR allele, leading to generation of AR-Vs. The study presented a case in which tumors from multiple metastatic sites in the same patient showed similar complex patterns of deletion and duplication within the AR LBD that explained the dominant expression of ARv567es [60]. However, due to the limitation of short-read sequencing and the involvement of target enrichment, this model may need further validation with long-read genomic sequencing.

Role of RNA splicing.

Although CRPC-specific splicing factors involved in the process have not been definitively identified, studies focusing on factors that regulate AR splicing have identified several key factors related to the RNA spliceosome. On the basis of predicted intronic/exonic splicing

enhancer sequences in cryptic exon 3 (CE3) of the AR gene, ChIP and RNA-pull down assays were performed and identified U2AF65 (splicing factor U2AF 65 kDa subunit, aka U2AF2, U2 small nuclear RNA auxiliary factor 2) and ASF/SF2 (alternative splicing factor 1/splicing factor 2) as two key factors mediating AR-V7 splicing [65]. A general splicing factor HNRNPA1 (heterogeneous nuclear ribonucleoprotein A1) was also reported to regulate AR-V7 expression in prostate cancer cells. Activation of NFκB2/p52 and c-Myc signaling resulted in recruitment of HNRNPA1 to the splice site of AR pre-mRNA to promote AR-V7 expression [71].

The ADT-induced lncRNA PCGEM1 (prostate cancer gene expression marker 1) was also implicated in AR-V7 generation. PCGEM1 regulates the binding of splice factors HNRNPA1 and U2AF65 to AR pre-mRNA [72]. In addition, p54/nrb (nuclear RNA-binding protein, aka NONO, non-POU domain-containing octamer-binding protein), a nuclear protein with multiple functions in RNA splicing and gene regulation positively regulates PCGEM1 expression, as revealed by DNA-pull down assay using PCGEM1 gene promoter in prostate cancer cells [73]. Suppression of p54/nrb by siRNA or a natural compound from cruciferous vegetables that interferes with p54/nrb-DNA binding resulted in reduced expression of PCGEM1 and AR-V7 [73].

Natural spliceosome inhibitors, thailanstatins (TST-A or TST-D), were used to investigate the relationship between AR-V7 and splice factors SAP155 (spliceosome-associated protein 155, aka SF3B1, splicing factor 3b subunit 1) and U2AF65. TSTs interrupts the interaction between U2AF65 and SAP155 leading to reduced binding to the polypyrimidine tract located between the branch point and the 3’ splice site, and consequentially reduced AR-V7 expression [74].

AR-V7 may be regulated by Jumonji domain containing 1A (JMJD1A, aka KDM3A), a histone demethylase that removes the repressive H3K9 methylation marks (H3K9me1 or H3K9me2) [75]. KDM3A also interacts with AR as an AR coactivator to regulate prostate cancer cell proliferation and survival by altering AR transcriptional program and elevating c-Myc levels [76–78]. KDM3A promotes alternative splicing of AR-V7 by binding to guanosine(G)-tract sequences in cryptic exon 3 (CE3) of the AR gene leading to recruitment of heterogeneous nuclear ribonucleoprotein F (HNRNPF) and other splice factors such as U2AF265 [75].

DDX39 [DEAD (Asp-Glu-Ala-Asp) box polypeptide 39] is an ATP-dependent RNA helicase implicated in RNA splicing, mRNA export, and telomere structure integrity. Using a shRNA library focused on 88 spliceosome-related genes, DDX39B was found to be a regulator of AR-V7 expression [79]. After Knockdown of DDX39B and its paralogue DDX39A in AR variant-expressed cells, the AR-V7 mRNA was selectively downregulated [79].

LIN28 is an RNA-binding protein involved in AR and c-Myc signaling in prostate cancer. Overexpression of LIN28 in PCa cell lines resulted in increased AR splice variant expression and resistance to anti-androgens. Downregulation of LIN28 can re-sensitize PCa cells to enzalutamide treatment [80].

The long non-coding RNA metastasis associated lung adenocarcinoma transcript 1 (MALAT1) was also implicated in regulating AR-V7 activity. Using enzalutamide-resistant prostate cancer cell lines, AR-V7 and MALAT1 were both elevated when AR signaling is suppressed. AR negatively regulates MALAT1 by direct binding to AREs in the promoter of MALAT1. Increased MALAT1 during AR suppression by enzalutamide could elevate AR-V7 transcription through interaction between MALAT1 and serine/arginine rich splicing factor 1 (SRSF1, aka ASF/SF2). CRPC progression in animal models was suppressed following MALAT1 knockdown [81].

Other co-factors regulating AR-V expression.

Protein kinase pathways may regulate AR-V expression. The most relevant is the AKT pathway, which modulates AR function and mediates survival signaling in CRPC. In a screen of kinase inhibitors involving a 145 small-molecule compound library and a high-throughput siRNA-kinome library, several kinases including Akt, Abl, and Src family kinases (SFK) were found to regulate AR-V7 mRNA expression and protein nuclear translocation. Following treatment of CWR22Rv1 cells with a Src/Abl dual kinase inhibitor PD180970, AR-V7 protein was decreased and cell proliferation inhibited in the absence of DHT. In the presence of DHT (when AR-FL is activated), the potency of PD180970 was decreased. Pre-treatment with AR-FL antagonist bicalutamide re-sensitized CWR22Rv1 cells to PD180970 with a substantial EC50 drop (from 184.2 to 12.6 nM) in the presence of DHT [82], suggesting that PD180970 reduced AR-V7 protein level and CWR22Rv1 growth in AR-independent manner. Tyrosine kinases such as ACK1/TNK2 regulate AR gene transcription through epigenetic modification. ACK1/TNK2 phosphorylates histone H4 at tyrosine 88 upstream of the AR transcription start site, and treatment with an ACK1 inhibitor (R)-9bMS reduced AR and AR-V7 levels and suppressed CRPC tumor growth [83].

Y-box-binding protein 1 (YB-1) is a transcription factor that binds Y-box (5′-ATTGG-3′), and also a RNA-binding protein involved in RNA splicing. In a study involving kinome arrays, Akt, RSK (ribosomal S6 kinase), ERK (extracellular signal-regulated kinases, p42/44 MAPK) were found to phosphorylate YB-1 and affect AR-V7 expression [84]. Treatment of CWR22Rv1 cells with kinase inhibitors against Akt, MEK (MAPK/ERK kinase, an upstream activator of MAPK/ERK) resulted in reduced levels of phosphorylated-YB-1, AR-FL, and AR-V7. However, an inhibitor of RSK specifically downregulated AR-V7 but not AR-FL. How RSK/YB-1 signaling affects AR splicing remains largely uncharacterized [84].

The relationship of AKT signaling and prostate cancer was evaluated by a synthetic AKT inhibitor alkyl-lysophospholipid edelfosine (ET-18-O-CH3). Edelfosine interacts with lipid rafts on plasma membrane, and induce endoplasmic reticulum (ER) stress following inhibition of phosphatidylcholine biosynthesis. Treatment of LNCaP and VCaP cells with Edelfosine resulted in suppression of AR-V7 expression and cell apoptosis that was enhanced by ATF3 (activating transcription factor 3), a corepressor of AR [85].

Other nuclear receptors may regulate AR-FL/AR-V7 expression. For example, ROR-γ (RAR-related orphan receptor gamma) recruits nuclear receptor coactivator 1 and 3 [NCOA1 and NCOA3, also known as steroid receptor coactivator (SRC)-1 and SRC-3] to an AR-ROR response element (RORE) to stimulate AR gene transcription [86]. ROR-γ

antagonists suppress the expression of both AR and its variant AR-V7 in PCa cell lines and tumors [86]. Given that many ROR-γ inhibitors are approved for treating autoimmune disease, it may be possible to repurpose these drugs to suppress AR-V7-driven prostate cancer.

Non-coding circular RNA (circRNA) or miRNA could also regulate AR-V7 expression. By *in silico* analysis, a circular RNA 17 (circRNA17, hsa_circ_0001427) was found to interact with microRNA 181c-5p (miR-181c-5p) leading to suppression of AR-V7 expression. By interacting with and stabilizing miR-181c-5p, circular RNA 17 increased the relative level of miR-181c-5p, which bound to the 3'UTR of AR-V7 transcript to downregulate AR-V7 expression. Castration can suppress circRNA17 expression by inhibiting its host gene *PDLIM5* (PDZ and LIM domain 5) expression via androgen response element (ARE) in the gene promoter. In this study, the level of circRNA17 was downregulated in clinical prostate cancer specimens especially in higher Gleason score prostate cancer specimens [87].

IV. Genomic functions mediated by AR-Vs.

As transcription factors, AR-Vs may mediate their downstream genomic functions by DNA binding and interaction with co-regulators following nuclear localization. While AR-FL and many AR-Vs may share common chromatin binding sites, genomic binding sites and transcriptional programs specific to AR-V7 have been reported [64,88–91]. In studies relevant to this topic, it is critical to take into consideration a few premises: 1) AR-V7 protein expression is specific to CRPC and negatively regulated by androgen signaling mediated by AR-FL [64,68]; 2) AR-V7 expression is often associated with a progressive phenotype under low androgen conditions [64,92]; and 3) AR-V7 often co-exists with AR-FL, and whether AR-V7/AR-FL forms heterodimer under low androgen conditions will affect interpretation of data [68,89,93–95].

Since our last review in 2016 [24], a few important factors mediating downstream functional effects of AR-V7 have been characterized. Chen et. al. revealed diverse AR-V7 cistromes and transcriptomes in different CRPC cell lines and clinical specimens [89]. Employing a high-resolution ChIP-exonuclease sequencing (ChIP-exo) approach using an AR-V7-specific antibody, HOXB13 was found to co-localize with AR-V7 and function as an essential co-activator mediating AR-V7 function. By inhibiting HOXB13 in AR-V7-expressing cells, the oncogenic function of AR-V7 was suppressed [89]. These results implicated that HOXB13, which is very specifically expressed in tissues of prostatic origin, could be an alternative target in suppressing the development of AR-V7-driven CRPC. Cai et. al. used specific antibodies against either AR-FL or AR-V7 for ChIP-seq. In this study, 15,162 out of a total of 17,409 binding sites were shared by both ARs, and only a small proportion of binding sites (about 12.8%, 2,221 out of 17,409 spots) were specifically bound by AR-V7. In these AR-V7-specific binding sites, ZFX (zinc finger protein X-linked) was found to exclusively colocalize with AR-V7. The AR-V7-specific binding sites are mainly located at the gene promoter and these AR-V7 targeted genes were mainly involved in MYC-bound genes or genes related to cell cycles and autophagy [88].

V. Preclinical and early clinical development of agents targeting AR/AR-Vs

Given the availability of blood-based detection methods and the general poor prognosis of patients having positive CTC AR-V7 testing results, recent efforts have focused on development of agents that may overcome drug resistance and poor prognosis by targeting AR-V7. Several compounds with *in vitro* anti-AR-V7 activity have already been evaluated in clinical trials, while many others are in various stages of preclinical evaluation and development (see companion review). A comprehensive review of the current literature on possible preclinical agents and strategies returned many studies. Due to space limitations, we summarized these studies in Figure 3 and Table I (with references).

VI. Conclusions

Laboratory investigations and findings reported since 2016 continue to support AR-Vs as biomarkers and therapeutic targets in prostate cancer. These new findings further validate the importance of AR-Vs, AR-V7 in particular, in castration-resistant prostate cancer. Development of mature measurement methods have enabled detection of the AR-V targets in both liquid and tissue specimens, and quantitative measurement data on AR-V7 support its functional and clinical relevance. While AR-Vs (e.g. ARv567es) driven by complex AR GSRs, as well as dominant expression of AR-V7 in isolated cases provide compelling examples of clonal expansion consistent with a driver role for AR-Vs in castration resistance, in most cases AR-FL continue to co-exist with AR-Vs, justifying further investigations to dissect the distinct roles of the different AR molecules. In this regard, distinct genomic functions mediated by AR-FL and AR-Vs have been defined in greater details now, further supporting the therapeutic relevance of AR-Vs. Given the feasibility of conducting blood-based detection for AR-V7, the hope is that clinical development of agents possessing anti-AR-V activity can be accelerated, even in the presence of many competing mechanism that may co-exist. Going forward, both challenges and opportunities exist in targeting AR-Vs to overcome resistance to current AR-directed therapies. Development of agents with specific anti-AR-V7 activity remains a top priority in prostate cancer research.

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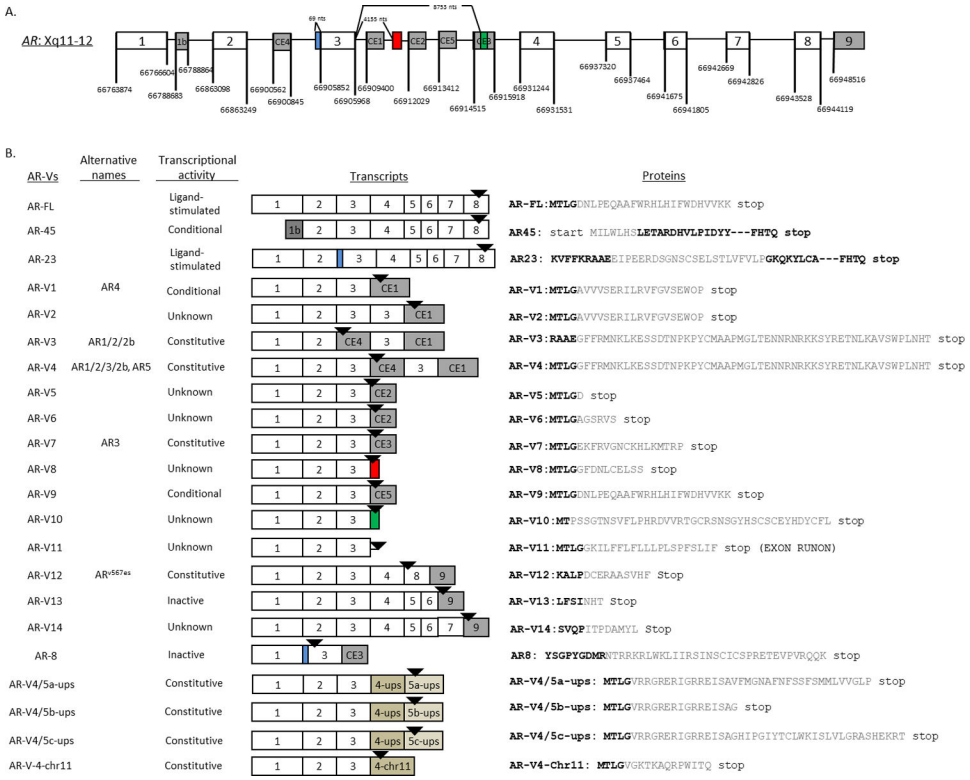

Figure 1.

Decoding the androgen receptor splice variant transcripts. (A) AR gene structure with canonical and cryptic exon splice junctions marked according to GRCh37/hg19 human genome sequences (not drawn to scale); (B) Nomenclature, functional annotation, exon compositions, and variant-specific mRNA (color matched to Figure 1A) and peptide sequences (in gray). Modified from reference #70.

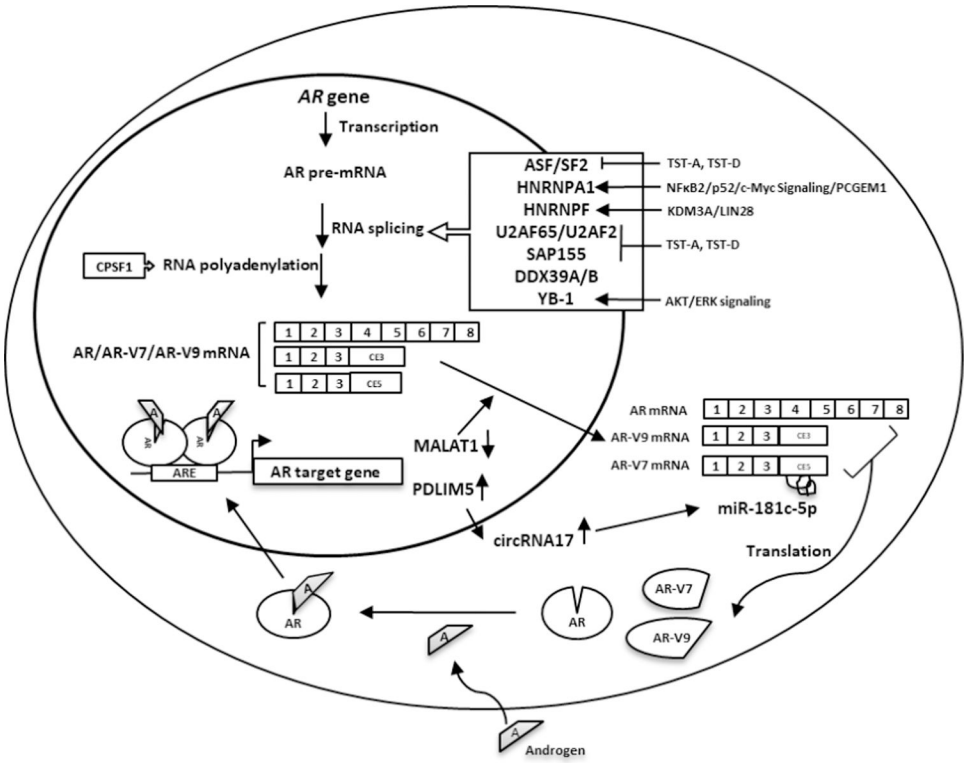

Figure 2.

Regulatory mechanisms involved in AR-V expression. Splicing factors involved in AR-V generation include U2AF65, ASF/SF2, HNRNPA1 (NFκB2/p52- and c-Myc-signaling related), and SAP155. LncRNAs involved in splice factor binding to AR pre-mRNA include PCGEM1 (p54/nrb signaling related) and MALAT1. Epigenetic modifier KDM3A may bind AR pre-mRNA and recruit HNRNPF and U2AF265. RNA helicase DDX39A & DDX39B and RNA-binding protein LIN28. Inhibition of CPSF1 by siRNA with morpholinos targeting a single polyadenylation signal (PAS) in AR CE3 suppresses AR-V7/V9 expression. Several protein kinase pathways, e.g. AKT/ERK/YB-1, and non-coding RNAs including circRNA17 and miR-181c-5p may also be involved in AR-V expression. Refer to text descriptions for details.

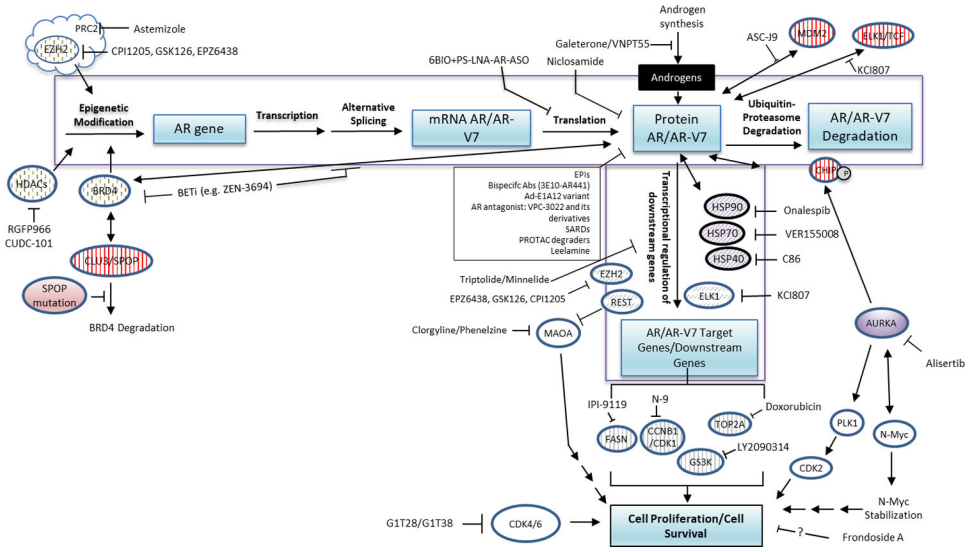

Figure 3.

Novel preclinical agents that suppress AR/AR-Vs by regulating gene expression, degradation, AR transcriptional activity, and downstream signaling. The following broad categories are summarized. Refer to Table I for details.

(1) Targeting AR/AR-Vs translation: 6BIO+PS-LNA-AR-ASO

(2) Novel AR antagonists: EPIs, 3E10-AR411, VPC-3022, SARDs, Ad-E1A12 variant,

(3) Enhancing AR or AR-Vs degradation: Niclosamide, ASC-J9, alisertib, PROTAC degrader, leelamine

(4) Targeting AR chaperones: C86, VER155008, onalespib

(5) Targeting molecules involved in epigenetic modification: BETi, HDACi, CUDC-101, EZH2i, astemizole

(6) Targeting AR/AR-Vs co-regulators or transcriptional activity: KCI807, triptolide/minnelide, clorgyline/phenelzine, IPI-9119, BETi

(7) Targeting AR/AR-Vs downstream signaling molecules: LY2090314, N9 + doxorubicin, G1T28/G1T38, alisertib, FrA

Table I.

Novel agents targeting AR/AR-V signaling

Name of the agents	Description of agents and their background	Action mechanisms
Agents inhibit androgen synthesis/AR antagonist/AR or AR-V degradation		
Galeterone and its derivatives VNPT55	Galeterone (TOK-001) and its analog VNPT55,a 3β-carbamate analog	• Multi-targeted steroidal agents that function as 17α-hydroxylase/17, 20-lyase A1 (CYP17A1) inhibitor •AR/AR-V antagonist • Post-translation modulators of AR degradation by enhancing ubiquitination [96]
EPI-001 compound family	Derived from endocrine disruptor bisphenol A, including EPI-001, EPI-002, EPI-506 (a prodrug of EPI-002, aka ralaniten acetate), EPI-7386	• Interact with the transcriptional activation domains TAU1 and TAU5 in AR N-terminal domain (NTD) to exhibit anti-androgen effects in preclinical studies [97]. • Selectively activate peroxisome proliferator-activated receptor-gamma (PPAR-γ) in prostate cancer cells [98]. • EPI-002 was able to inhibit proliferation of prostate cancer cell lines resistant to enzalutamide and docetaxel with unknown mechanisms [99]. • EPI-7386 is a more potent, orally bioavailable and metabolically stable AR NTD inhibitor that appears to be a better drug candidate than its predecessors, and possess antitumor activity in several AR-V7-positive CRPC cell lines and xenograft models including in enzalutamide-refractory models [100].
3E10-AR441	A bispecific single-chain variable antibody contains an anti-DNA monoclonal antibody (3E10) to facilitate cell penetrance, and an anti-AR N-terminal domain mAb (AR441) generated with epitope aa299–315 of the AR protein.	3E10-AR441 may enter cell nucleus and bind to AR and AR-V7 and block their function [101].
AR degradation enhancer ASC-J9	Aka dimethylcurcumin, 5-hydroxy-1,7-bis(3,4-dimethoxyphenyl)-1,4,6-heptatrien-3-one	• Promoting AR degradation by increasing its association with MDM2 and phosphorylation of AKT and MDM2 to promote the proteasome-dependent AR degradation [102]. • Increasing the dissociation of AR with selective co-regulators protein 55 (ARA55) and ARA70, which subsequently resulted in suppression of AR transactivation and AR-mediated cell growth [102]. • Decreasing CD133(+)-stem/progenitor PCa cell population in cell culture and suppress CD133(+)-PCa cell invasion via inhibition of known AKT-enhancer of zeste 2-signal transducer-activator of transcription 3 (AKT-EZH2-STAT3) axis involved in regulating stem-like tumor cells [103]. • Overcome the resistance of enzalutamide in prostate cancer cells by decreasing MALAT-1-induced AR-V7 protein level [81].
Novel AR antagonists: VPC-3022 and its derivatives VPC-3033 and VPC-3045	VPC-3022: 10-benzylidene-10H-anthracen-9-one, and its derivatives (e.g. VPC-3033, VPC-3045)	• VPC-3022 docks at the hormone binding site of AR. • May induce AR/AR-V7 degradation though the unknown mechanism [104,105].

Name of the agents	Description of agents and their background	Action mechanisms
Selective AR degraders (SARDs): UT-69, UT-155, and (R)-UT-155; modified indolyl and indolinyl propanamides (series II and III); bisphenol AP	A series of modified AR antagonist molecules (including UT-69, UT-155, (R)-UT-155, indolyl (series II) and indolinyl (series III) propanamides)	• Targeting AR as antagonist and functioning as AR/AR-V degrader. • The SARD activity may be mediated through a binding site in the NTD of AR/AR-Vs, implicating its capability in overcoming drug resistance mediated by AR LBD point mutation or AR-Vs [106,107].
Proteolysis targeting chimeras (PROTAC) degraders: ARV-110, ARD-69, ARCC-4, ARV-771	Bifunctional small molecules which mainly combine a target-binding moiety and an E3 ligase recruiter to bring a target protein into contact with an E3 ligase for ubiquitylation and subsequent protein degradation by proteasome [108]. ARV-110, ARD-69, ARCC-4: Function against AR [109–112]. ARV-771: Function against AR co-regulators of transcriptional activity bromodomain and extra-terminal (BET) family proteins BRD2/3/4 [113].	• Inducing AR or its co-regulator degradation. • Inhibiting AR signaling including suppression of AR-FL and AR-V7 expression [113]. • On March 2019, the first phase I clinical trial on AR PROTAC degrader ARV-110 has been launched by Arvinas Inc. in multiple clinical institutes with primary objective in evaluating the safety and tolerability in men with mCRPC who have progressed on at least 2 prior systemic therapies for their castrate resistant disease (one of which must be enzalutamide or abiraterone) (NCT03888612).
Antisense oligonucleotide enhancer: 6BIO	6-bromo-indirubin-3'-oxime (6BIO), a derivative of indirubin, was found to enhance the activity of antisense oligonucleotides (ASOs)	• Repressing AR-FL and AR-V7 expression through the simultaneous inhibition of GSK-3α and GSK-3β. • The combination of 6BIO with an anti-AR phosphorothioate (PS) modified locked nucleic acids containing ASO (PS LNA AR-ASO) virtually eliminated all AR expression in PCa cells [114].
Reluzole	An anti-glutamatergic agent and FDA-approved treatment for amyotrophic lateral sclerosis (ALS)	Promoting protein degradation of AR-FL, mutant ARs, and AR-V7 via activating transcription factor 6 alpha (ATF6α)/inositol requiring kinase enzyme 1 alpha (IRE1α)-mediated endoplasmic reticulum stress (ERS) pathway and downstream selective autophagy [115].
Leelamine (LLM)	A component extracted from pine tree bark	Non-covalently interacting with Y739 in AR and inhibits AR activity [116].
Targeting AR chaperones		
Heat shock protein (HSP40/70/90) inhibitors	HSP40 inhibitor: C86 (3-nitro-2',4',6'-trimethoxychalcone) HSP70 inhibitors: Quercetin and VER155008 HSP90 Inhibitor: onalespib (AT13387)	• C86: identified by a compound screen for perturbing the AR-FL/AR-V7 transcriptional program in 22Rv1 cells. Decreased protein levels of AR-FL and AR-V7 with suppressed FL-AR- and AR-V7-mediated transcriptional activity. The inhibition effect of C86 on tumor growth was enhanced by combination administration of HSP70 inhibitor JG98 in a 22Rv1 CRPC xenograft model [117]. • HSP70 inhibitors: quercetin and VER155008 decreased LNCaP95 cell proliferation, and the levels of AR-FL and AR-V7. VER155008 decreased AR-FL and AR-V7 in mRNA level indirectly via down-regulation of Y-box binding protein 1 (YB-1) phosphorylation and nuclear localization [118]. VER155008 was also reported to destabilize AR-V7 protein by promoting AR-V7 dissociation from HSP70 and degradation via co-chaperone/E3 ubiquitin ligase STIP1 homology and U-box containing protein 1 (STUB1)-related ubiquitination [118]. • HSP90 Inhibitor onalespib

Name of the agents	Description of agents and their background	Action mechanisms
		(AT13387): this second generation HSP90 inhibitor potently inhibited the growth of AR-V7-containing cell lines VCaP, 22Rv1 and LNCaP95 *in vitro*. Onalespib also decreased protein levels of AR and AR-V7, together with other client proteins. Onalespib also down-regulated mRNA level of AR-V7 but not AR-FL via possible disruption of AR splicing following HSP90 inhibition [119]. However, this agent did not demonstrate efficacy in a Phase I/II trial when combined with abiraterone in patients progressing on abiraterone (NCT01685268).
Targeting molecules involved in epigenetic modification		
BET/BRD4 inhibitors: GSK525762, GS5829, OTX015, JQ1, ABBV-075, ZEN-3694, PFI-1	The bromodomain and extra-terminal (BET) domain containing protein 4 (BRD4), an epigenetic adapter, interacts with the acetylated histones 3 and 4 through its bromodomain, and regulates gene transcription by recruiting and activating the essential transcription elongation complex, the positive transcription elongation factor b (P-TEFb). BRD4 is also an AR transcriptional coregulator. BRD4 interacts with the AR NTD to facilitate transcriptional activity [120,121]. BET inhibitors (BETi) (e.g. GSK525762, GS5829, OTX015, JQ1, ABBV-075, ZEN-3694, PFI-1) targeting BET-containing protein family members including BRD2, BRD3, and BRD4 were under development for CRPC treatment.	• Disrupting BRD4-AR interaction and prevent DNA binding of AR-FL or AR variants. • Decreasing AR-V7 expression by regulating splicing factors required for its generation [120,121]. • Enhancing the response of homologous recombination-proficient cancer cells to PARP inhibition (PARPi)-induced DNA damage by decreasing transcription of critical proteins BRCA1 and RAD5 involved in homologous recombination of double-strand DNA repair [122]. • BRD4 protein degradation was depended on cullin 3-speckle-type POZ protein (CUL3-SPOP)-mediated ubiquitination. In PCa cells with SPOP mutation, BETi treatment may lack efficacy due to the dominant-negative effect of mutant SPOP and an increased level of BRD4, implicating that SPOP mutation detection may be considered as a treatment selection biomarker for BETi treatment [123,124]. ZEN-3694 is a BETi that has entered clinical trials either as a single agent Phase I study in CRPC (NCT02705469) or a Phase 1b/2a safety and tolerability study in combination with enzalutamide in mCRPC patients (NCT02711956).
HDAC3-selective inhibitor: RGFP966	A slow-on/slow-off, competitive, tight-binding inhibitor targeting class I HDACs (HDAC1, 2, 3, 8) with the greatest inhibition of HDAC3 [125].	Epigenetically suppressing AR signaling without induction of epithelial-mesenchymal transition (EMT) in PCa cells [126].
EZH2 inhibitors: GSK126, GSK343, GSK503, NPD13668, EPZ6438 and PRC2 inhibitor: astemizole	Enhancer of zeste 2 (EZH2), the enzymatic subunit of polycomb repressive complex 2 (PRC2), catalyzes histone H3 lysine 27 trimethylatiion (H3K27me3) to trigger gene silencing. In prostate cancer, EZH2 was found to be overexpressed and quantitatively associated with progression and poor prognosis [127]. In addition to its methyltransferase activities, EZH2 could also act as transcriptional coactivator in gene activation including AR [128].	• EZH2 binds to AR gene exon 1 around ~1.7–2.5 kb downstream of the transcriptional start site (TSS) to elicit its transactivity in a PRC2-independent manner, and thus mediates dual methylation-dependent and -independent transcription programs in PCa [129]. • Preclinical study showed that using EZH2 inhibitors (such as GSK126, GSK343, GSK503, NPD13668) can effectively inhibit the growth of prostate cancer cells [130,131]. • EZH2 inhibitors GSK126 and EPZ6438 could also help to restore the expression of AR and re-sensitize tumor response to enzalutamide in neuroendocrine prostate cancer (NEPC) mouse models with Pten+Rb1 KO or Pten+Rb1+Trp53 KO [132]. Together with docetaxel, the resistance of EZH2 inhibitor could be overcome in PTEN-mutated cancer cells via releasing EZH2 suppression on PTEN effector FOXO1 gene expression and

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		sustaining FOXO1 nuclear localization to induce cell death [133]. • EZH2 and embryonic ectoderm development (EED) (another component of PRC2) could also affect AR signaling by directly interacting with AR to target AR downstream genes. Using a newly identified PRC2 inhibitor astemizole, the AR-EZH2-EED interaction can be disrupted and led to protein degradation of AR and EZH2; thus astemizole exerted a potent therapeutic effect on prostate cancer preclinically [134]. • Currently, the EZH2 inhibitor CPI-1205 (Constellation Pharmaceuticals) is under Phase 1b/2 study in mCRPC patients to determine the maximum tolerated dose and safety in combination with enzalutamide or abiraterone/prednisone (NCT03480646) and AR-V7 expression in CTC was included as a monitoring variable for drug response.
CUDC-101	CUDC-101 exerts inhibition effect on multiple targets including histone deacetylase, the receptor kinases epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2) in lung and breast cancer cells [135].	Suppressing PCa cell growth and downregulated AR-FL and AR-V7 via HDAC5 and HDAC10 inhibition [136].
CBP/p300 inhibitors: GNE049, CCS1477, NEO2734	The highly homologous histone acetyltransferases CBP [cAMP response element binding protein (CREB) binding protein] and p300 are both coactivators of AR, especially the p300 is widely required for androgen-induced gene regulation and histone acetylation and methylation [137]. CBP and p300 were both highly expressed during androgen deprivation in prostate cancer [138].	• Several CBP/p300 bromodomain inhibitors (e.g. GNE049, CCS1477), and a novel dual inhibitor of both BET and CBP/p300, NEO2734 (Epigene Therapeutics Inc.) had been suggested in suppression of prostate cancer growth in models of *in vitro* and *in vivo* [139–141]. • In preclinical study, CCS1477 led to protein reduction of AR-FL, AR-Vs, and c-Myc, AR-targeted gene expression, and *in vivo* tumor growth of bicalutamide-resistant LNCaP cells either as monotherapy or in combination with enzalutamide [140]. • CCS1477 is currently in a Phase I/IIa study for CRPC by CellCentric Ltd (NCT03568656) [142].
Targeting AR/AR-V co-regulators or related downstream signaling		
Cell cycle CDK4/6 inhibitors: G1T28 and G1T38	G1T28 and G1T38 were two new CDK4/6 inhibitors that were previously investigated in lung and breast cancers (e.g. NCT02978716, NCT02514447). PCa is also a cancer with upregulated expression of cyclin D1 and activation of the G1 cyclin-dependent kinases CDK4/6 [124].	Stice et. al. tested G1T28 and G1T38 in preclinical *in vitro* and *in vivo* models of PCa as single drug and compared them to docetaxel and enzalutamide. Results showed these inhibitors were as effective as docetaxel in mouse models of enzalutamide-resistant LNCaP-AR-F876L+ xenograft or AR-V7+ CWR22Rv1 xenograft with less toxicity [143].
Aurora kinase A inhibitor: alisertib	Aurora kinase A (AURKA) is a serine/threonine kinase with a major role in promoting mitosis by phosphorylating polo like kinase 1 (PLK1) for CDK1 activation. AURKA could also stabilize the transcription factor N-MYC by preventing its proteasomal degradation, and thereby promotes G1–S progression.	• Inhibition of AURKA suppressed the growth of CRPC cells that had high AR expression, and AURKA also indirectly regulated the expression of AR-V7 in PCa cells [144]. • Inhibition of AURKA reduced AR-V7 protein level and AR target gene expression and suppressed CWR22Rv1 cell proliferation *in vitro* [145]. •

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		AURKA inhibition decreased AR proteasome degradation via decreasing phosphorylation of E3 ubiquitin-protein ligase CHIP (carboxyl terminus of Hsc70 interacting protein) [146]. • Alisertib is under a phase II trial for mCRPC patients with neuroendocrine/small cell PCa feature (NCT01799278). Eight patients (13.4%) were radiographic progression free at 6 months as primary endpoint. Eighteen patients (30%) had stable disease or better at cycle 3 scans with exceptional responders were identified including 2 with complete remission of liver metastases on therapy and 2 with prolonged stable disease for14 months and 3.8 years. Although the study did not meet its primary endpoint, a subset of patients of mCRPC with activated signaling in Aurora-A and N-myc achieved promising clinical response [147].
Triptolides and its pro-drug minnelide	Triptolide (TPL) is a diterpene triepoxide isolated from a Chinese herb Tripterygium wilfordii	Triptolide inhibited the proliferation of prostate cancer cells via variable mechanisms, most notably through inhibition of gene transcription [148]. However, due to its poor aqueous solubility, its clinical application was limited and a water-soluble pro-drug minnelide was developed [149]. Preclinically, minnelide showed sufficient effect on suppressing 22Rv1 tumors growth in mice [150].
ETS Like-1 protein (ELK1)-AR interrupter: KCI807	ELK1 is a member of the E26 transformation-specific (ETS) family of transcription factors and of the ternary complex factor (TCF) subfamily. Phosphorylation of ELK1 by MAPKs activates TCF and enhances ELK1-containing TCF binding to the corresponding DNA response element. ELK1 could also interact with AR and activate some AR targeted genes [151]. Using cell-based systems, KCI807 was discovered as the top hit of 92 compounds screened out from a total of 18,270 compounds.	• Disrupting AR interaction with ELK1 • Reducing chromatin recruitment mediated by ELK1 • Suppressing growth of enzalutamide-resistant prostate cancer cells overexpressing AR-V7 [152].
Monoamine oxidase A (MAOA) inhibitors: clorgyline and phenelzine	**Clorgyline**: a selective, irreversible MAOA inhibitor **Phenelzine**: a non-selective, irreversible MAOA inhibitor	• Clorgyline and phenelzine decreased growth and proliferation of androgen-sensitive LNCaP cells and castration-resistant prostate cancer cells C4-2B, and 22Rv1. • Clorgyline also inhibited expression of AR-FL and AR-V7 expression and decreases growth of an enzalutamide-resistant, AR-V7-positive C4-2B cell line [153].
Fatty acid synthase (FASN) inhibitor: IPI-9119	IPI-9119 irreversibly inhibited FASN	Reducing FASN activity by IPI-9119 inhibited PCa cell growth with cell apoptosis and altered metabolome. The altered metabolome might subsequently induced reticulum endoplasmic stress response that led to protein translation inhibition including AR/AR-V7 protein [154].
GSK3 inhibitor LY-2090314	LY-2090314, a GSK3 inhibitor, identified as AR-V7 transcription inhibitor using a PSA-luciferase reporter system with AR-V7 co-expression	LY-2090314 inhibited AR-V7 action partially via the activation of β-catenin signaling. Reciprocally, AR-V7 signaling also negatively regulated β-catenin signaling, and therefore, GSK3 inhibition can repress AR-V7 transcriptional activity by inducing a positive feedback to maintain the activation of β-catenin signaling [155].
CCNB1/CDK1 inhibitor: N9-isopropylolomoucine (N-9)	With an integrated and unbiased data mining and experimental strategy, Magani el. al. identified a 7-gene set [(kinesin family member 20A (KIF20A), kinesin family	By combined treatment of doxorubicin (DOX), a TOP2A inhibitor, and N9-isopropylolomoucine (N-9), AR-V7 downstream cell cycle genes were targeted and led to the reduction of cell proliferation in CRPC cell line 22Rv1 [156].

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	member 23 (KIF23), topoisomerase DNA II alpha (TOP2A), cyclin B1 (CCNB1), cyclin B2 (CCNB2), BUB1 mitotic checkpoint serine/threonine kinase (BUB1)]. These genes were upregulated with association of higher levels of AR-V7 in prostate cancer patients.	
GHR antagonist: pegvisomant	Growth hormone (GH)-insulin like growth factor 1 (IGF-1) axis signaling is associated with prostate cancer. The source of GH in PCa might come from pituitary or tumor itself as autocrine/paracrine since GH was detected in PCa cells. In LnCaP and enzalutamide-resistant C4–2B cells, GH promoted cell growth with increased expression of AR-V7 and IGF1.	GHR antagonist pegvisomant blocked GH action and reversed the effect of GH on PCa cells, which also implicating the promoting role of GH in PCa progression during castration [157].
VNLG-152	VNLG-125, a novel retinamide as retinoic acid metabolism blocking agent (RAMBA) with multi-function in anti-tumor therapy.	• Suppressing PCa tumor growth via reducing AR/AR-V7 protein levels and AR signaling pathway [158]. • Inhibiting PCa invasiveness by reducing epithelial-mesenchymal transition (EMT) and mitogen-activated protein kinase-interacting kinase 1/2 (MNK1/2)–eukaryotic initiation factor 4E (eIF4E) pathway [159].
Adenovirus 12 E1A (Ad-E1A12)	A protein identified in adenovirus contains a conserved region (CR3) that could compete with co-repressors for binding to nuclear receptors, thereby promoting nuclear receptor activation	The full length of E1A12 (266aa) preferentially binds to AR, while the shorter E1A12 variant (235 aa) interacts stronger with AR-V7 to promote AR nuclear translocation, trigger apoptosis and these functions were enhanced by PI3K-AKT-mTOR signaling pathway inhibition [160].
Artesunate	Artemisinin derivatives (ADs), such as artesunate, are semi-synthetic compounds derived from Artemisia annua and used for treating malarial infection	Artesunate (AS) was reported by Nunes et. al. that in combination with bicalutamide (Bic), it exhibited inhibition effect on prostate cancer cells by suppressing nuclear factor (NF)-κB signaling, reducing AR and AR-V7 levels via ubiquitin-mediated proteasomal degradation, and inducing oxidative stress and apoptosis [161].
Frondoside A (FrA)	A triterpene glycoside compound from sea cucumber	• Inducing PCa cell cycle arrest and apoptosis in caspase-dependent or -independent manner via up-regulating several pro-apoptotic proteins (Bax, Bad, PTEN), and down-regulating of anti-apoptotic proteins (survivin and Bcl-2). • Inhibiting pro-survival autophagy in PCa cells to inhibit growth of prostate cancer cells with high AR-V7 activity [162].